Determination of in-lab site fidelity and movement patterns of *Paruroctonus utahensis* (Scorpiones: Vaejovidae)

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Abstract. Many animals build homes to which they return after excursions. However, the sensory and motor mechanisms that animals use to home are poorly understood. Sand scorpions, including *Paruroctonus utahensis* (Williams 1968), make burrows from which they emerge to hunt at night. These scorpions spend most of their surface time within about a meter of their burrow. Our goal was to create a laboratory environment conducive to scorpion homing behavior. Specifically, our objectives were to verify in-lab burrow use similar to field observations and to characterize scorpion movements in these artificial environments. Tests occurred in circular, sand-filled arenas (65 cm diam); in the center of each was a shelter (a small jar lid with openings for the scorpions to enter). We used IR cameras to film all trials from above in a room with a 14:10 hour light-dark cycle. Animals were tested over a 7-day period for their tendency to establish and return to their shelters on a normal day/night cycle. Time-in-shelter percentages showed significant evidence of shelter use, consistent with their normal burrow use in the field. In the second experiment, we wrote a MATLAB program to automatically track several hours of videotaped scorpion nocturnal movements. Animals spent most of their time along the arena walls but made intermittent forays across the arena center. When they returned to their shelters, their movements appeared to be direct and deliberate. This behavioral set-up will be useful in future attempts to deduce the sensory information these animals use to return to their burrows.

Keywords: Homing, behavior, navigation, sensory, scorpion

Many species of animals build homes to which they repeatedly return, suggesting navigational capabilities. While humans use vision, extensive memory, and other methods to navigate, many animals rely on different means. For example, desert ants appear to retrace familiar visual paths learned during training excursions established initially by path integration (Collett & Collett 2000; Wehner & Srinivasan 2003; Wittlinger et al. 2006; Baddeley et al. 2012), some slugs use odors as clues that help them find their homes (Chelazzi et al. 1988), and fiddler crabs often use "kinesthetic short-range homing," a form of path integration, when near their burrows (Cannicci et al. 1999). Other arachnids, such as spiders, have also been shown to orient and navigate using path integration (Moller & Görner 1994; Ortega-Escobar 2002). One problem is that many of the typically studied homing animals (bees, pigeons, bats) travel great distances, making it difficult to track their exact long-range paths in the laboratory.

Homing behaviors in the desert grassland scorpion, *Paruroctonus utahensis* (Williams 1968), are less understood. These nocturnal animals are ideal for homing studies because they are abundant, easy to obtain, and live in conditions that can be simulated in the laboratory. Scorpions have several sensory structures that may be used during homing. Their eyes, located laterally and medially on the prosoma, are sensitive to light levels typical of a clear nighttime sky (Fleissner & Fleissner 2001). The tip of the tail is also sensitive to light (Zwicky 1970; Rao & Rao 1973). They have tarsal taste hairs (Foelix & Schabronath 1983) and sensitive, mid-ventral organs called pectines (Cloudsley-Thompson 1955; Foelix & Müller-Vorholt 1983; Gaffin & Brownell 1997, 2001; Wolf 2008), which could be used to detect chemical and textural cues around their burrows. They also have mechanosensory structures, including trichobothria (Hoffmann 1967; Messlinger 1987) on the pedipalps, which detect air currents and perhaps minute pressure changes around the burrow

entrance (Gaffin 2011). Mechanosensory tarsal hairs could be used to detect previously made footsteps around the burrow.

Observations of scorpion homing behavior are typically conducted in the field, but studies are constrained by time of year and weather. In field studies, desert scorpions showed site fidelity after displacements of up to 8 m from their burrows, and straight-line return paths when < 50 cm from their burrows. Further, it is not uncommon for scorpions to be faithful to the same burrow for time spans ranging from months to years (Polis et al. 1986). We wanted to develop a lab-based assay to facilitate year-round observation and allow maximal control of potential sensory cues. Our objectives in this study were to induce scorpions to adopt artificial shelters in laboratory arenas, and to document their nocturnal surface activities in these arenas. We found that scorpions' pattern of shelter occupation was consistent with their normal field behavior in light and dark conditions. The scorpions moved consistently during nighttime hours and spent the majority of their out-of-shelter time close to the arena wall. Nevertheless, we noted occasional departures from the wall toward the arena center, sometimes directly into the mock burrow.

METHODS

Animal collection and care.—The subjects of this study were twelve female *Paruroctonus utahensis*. Eight were collected outside of Monahans, TX in the springs of 2010 and 2011, and four were collected near the Sevilleta Field Station, NM in the fall of 2012. They were each maintained in 3.8 L glass jars containing sand to a depth of approximately 2.5–5.0 cm and a piece of clay pot. Diet consisted of one cricket every 2 weeks. We moistened the sand weekly with approximately 5 mL of water to hydrate the scorpions. The laboratory was kept between 24–27 °C and 65–70% RH, with a light-dark phase of 0400–1800 h light and 1800–0400 dark.
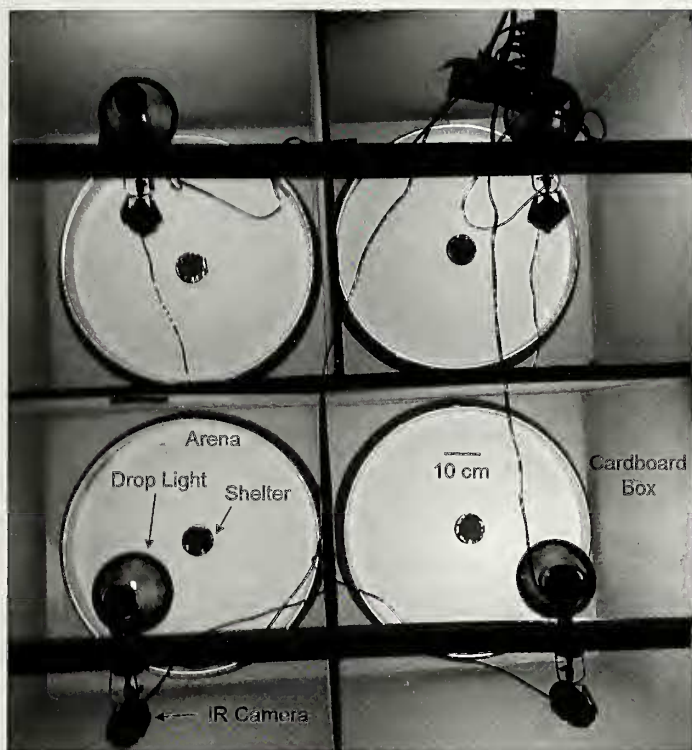
Figure 1.—Experimental apparatus. Scorpions are placed in circular arenas containing sand (2 cm deep) and an artificial shelter in the center. White clamp lights suspended above each arena simulate a normal light:dark cycle. Cameras are suspended above each arena to capture scorpion movements and emit IR light during the dark cycle for visibility. Cardboard boxes surround each arena to block light from adjacent arenas.

Arenas.—To approximate the sandy environments inhabited by the subjects, we set up four behavioral arenas, each consisting of a water heater drain pan (Camco, product #20813; 68.6 cm top diam., 65.4 cm base diam., 7.62 cm height). We covered the drainage hole (5-cm diam.) in the side of each pan with a strip of standard duct tape. The pans each contained 3.5 L of sand spread and leveled across the bottom (1 cm deep). We constructed shelters from two-part canning jar lids (Kerr home canning lid, 8.9 cm diam., 1.3 cm tall) from which three equidistant, 2.54 cm-wide slots were removed from the screw-on ring. The flat part of the lid was glued to the ring with Krazy Glue. Each shelter was placed on the sand in the middle of the arena. We placed the arenas in the bottoms of open-topped cardboard boxes (76.2 cm × 76.2 cm × 76.2 cm) to avoid shadow-cast on adjacent arenas. A droplight (Bayco 75-watt incandescent clamp light) was suspended 92.7 cm above the center of each arena. Each lamp contained an 8.6-watt bright white LED bulb (Ecosmart) (Figure 1). A dial timer switch controlled each light's operation on an approximated 14-hour on, 10-hour off cycle (lights off at 18:17, lights on at 04:16). We mounted one high-resolution infrared surveillance camera (Defender, 8-channel Smart Security DVR) next to each of the droplights (94 cm above arena). The camera videos were continuously captured on the DVR at four frames per second.

Site fidelity experiment.—Our experimental arenas were designed to supplement a previous experiment, where scorpi-ons were shown to travel towards their burrow location in the laboratory (Bost & Gaffin 2004) after displacement. In the current study, our goal was to gather baseline activity patterns while also developing software to track their precise, long-term movements in artificial habitats.

We conducted two trials with four scorpions per trial. We placed a recently fed scorpion (Monahans collection) in each of the four arenas for 8 hours of acclimation. After acclimation, the subjects were recorded continuously for 7 days. Once the first trial ended, we mixed and leveled the sand to create a clean, unmarked surface before the second trial began.

We reviewed the footage and scored scorpion locations at 84 pre-selected times: seven during each light phase and five during each dark phase of the weeklong period (proportionate to the 14:10-hour light-dark cycle). We selected observation times using the RAND function in Microsoft Excel, eliminating times occurring within 1 hour of a previously selected time. Scorpions received a score of "1" if they were under the jar lid, and "0" if they were not. Each animal in the first trial received a total of 84 scores (49 light and 35 dark). We gave 83 scores in trial two (49 light and 34 dark) as a result of a temporary pause in videotaping during one of the dark observation times.

We compiled time-in-shelter percentages for all eight scorpions and applied a 2-tailed, paired t-test (our data were normally distributed). We then compared the percentages in light and dark conditions with the α-value set at 0.05.

Location mapping experiment.—To trace scorpion paths, we placed four *P. utahensis* (Sevilleta collection) in the arenas after cleaning and leveling the sand, and recorded them overnight. We analyzed footage during the first 7 hours of the first dark cycle (16:25–23:25) to correspond with the scorpi-ons' normal peak activity period as seen in pilot studies using other *P. utahensis* scorpions. The DVR stored the video footage as several compressed files (each containing approximately one hour of footage). We converted these DVR compressed files (.264 format) into AVI files (AVI Generator 2.0) and finally to MP4 files (Converter Lite). We imported the MP4 files to iMovie (v. 9.0.9, Apple Inc.), split each file into two 30-minute segments, and increased speed to 2000% of initial footage. These files were then exported as .mov format (320 × 240 pixels) and imported into MATLAB (ver. R2012a, The MathWorks, Inc.).

Our MATLAB script used a frame-by-frame subtraction method to detect a scorpion's movement and plot its position in each video frame. We exported these coordinates to Excel. Occasional artifact coordinates were induced by poor video quality (such as from IR spotlighting and/or reduced resolution from converting video files through multiple formats). We categorized coordinates as artifacts if they met any of the following criteria: the point was outside the arena's radius; the point was more than 16 cm from the previous position; or the point was inconsistent with normal scorpion motion (e.g. a sharp 'spike' away from and then back towards a normal pathway). On other occasions, we identified artifacts by comparing suspiciously high position values with actual video footage.

After identifying and removing artifact coordinates, we created a macro that used the data to create a "movement density map" for each scorpion. The macro separated each
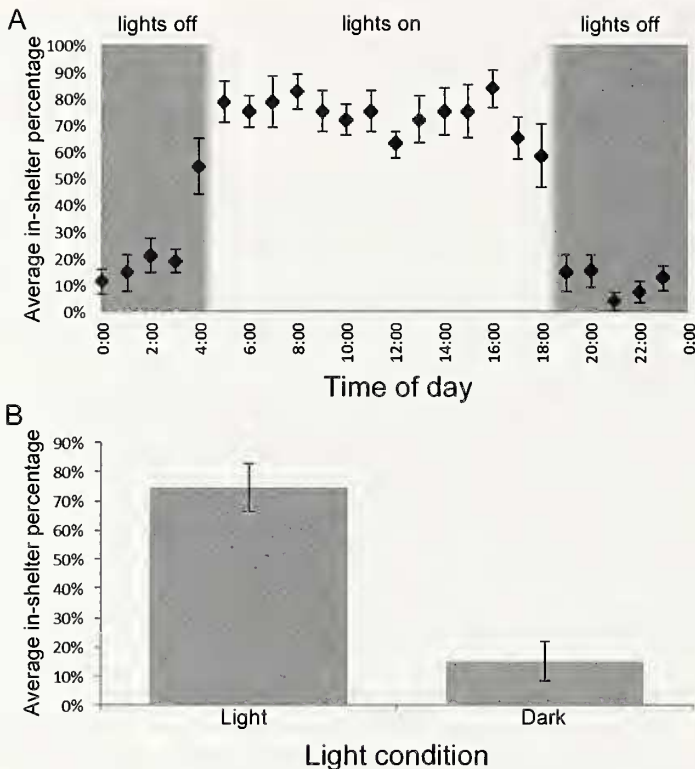
A

B

Figure 2.—A 10-day study indicates that scorpions show fidelity to artificial shelter sites in the lab. A) Average percentages of in-shelter observations at randomly selected times of the day. B) Average in-shelter percentages in "light" vs. "dark" conditions (mean ± SE).

arena into 0.648 cm² sections (derived from a 101 × 101 cell matrix applied to the image) and plotted the amount of time (in seconds) that the scorpion spent moving in each section. These figures did not account for times where subjects were still. All data for one of the four scorpions were removed due to the presence of a live cricket in the arena that affected the scorpion's behavior.

RESULTS

Site fidelity.—The apparatus in the site fidelity experiment (Figure 1) was used to determine if scorpions would use artificial shelters similarly to natural burrows in the field. We first compared the averages of all the "in" scores by hour over the 7-day span (Figure 2A; for example, the data point with the x-value of "1:00" represents the average of all in-shelter percentages within all of the 1:00–1:59 time frames over the 7-day period). Fidelity was determined by comparing "in" values in light vs. dark conditions (Figure 2B). In light conditions, the eight scorpions averaged 74.5% ± 8.2% (mean ± SE) of the observed times inside their artificial shelter; in dark conditions, they averaged 15.0% ± 6.8% (mean ± SE). This pattern was significantly different from random at $P = 0.001$ ($t = 7.403$, d.f. = 7).

Location mapping.—We made long-term observations of scorpion surface activity as a baseline for future behavioral assays. Figure 3 shows the nocturnal movements of three scorpions, as well as their corresponding movement density maps. All three scorpions showed extensive wall-walking behavior interspersed with periodic wall departures and arced

paths across the middle of the arena. The patterns of movement away from the walls were qualitatively different for the three scorpions. Scorpion 2 (Figure 3B) had the highest movement density in the arena center and made several off-wall excursions that retraced consistent elliptical paths. The off-wall excursions of scorpions 1 and 3 appeared more random.

The density maps show the scorpions' positions during their periods of movement. During the 420-minute observation period, scorpion 1 was moving for a total of 118 min (28.1%; Fig. 3A), scorpion 2 moved for 129 min (30.7%; Fig. 3B), and scorpion 3 moved for 124 min (29.5%; Fig. 3C). The scorpions also varied in the percentage of total movement spent along the wall (all movement within 3 cm of the wall was considered "wall movement;" everything else was considered "center movement"). Scorpions 1, 2, and 3 spent 92%, 54%, and 86% of their movement time along the wall, respectively. Movements in the arena center sometimes included activity at the shelter openings. Scorpion 2 spent 17% of its arena center movements partially inside of the shelter, moving just enough for detection by our software.

DISCUSSION

The animals in this study treated their canning lid shelters with site fidelity analogous to home burrow inhabitation patterns in their native environment (Polis et al. 1985). Furthermore, cycling of the room lights to simulate a day-night pattern appears to induce outside-shelter activity patterns similar to those in the wild.

We developed MATLAB scripts to automatically track scorpion movement patterns. While this process greatly increases the speed and efficiency of gathering long-term movement data, several modifications could improve the assay. First, the duct tape covering the pre-cut hole in the drain pan influenced animal behavior; when reviewing video footage, we observed our subjects attempting to crawl on the duct tape-covered surfaces. Future studies should use plugs to seamlessly close the holes or use arenas without pre-cut holes. Second, video image quality should be increased to reduce tracking artifacts. A light dispersion filter could reduce spotlighting and make the IR coverage of the arena more uniform, allowing for more accurate tracking during dark hours. Limitations imposed by the resolution of the surveillance system (coupled with additional losses imposed by file conversion) could be overcome by using a higher resolution system, but that would generate additional data storage demands. If we could directly extract and store movement data without saving the extraneous arena information, this issue could be mitigated.

This in-lab site fidelity assay provides a useful tool for addressing questions of scorpion homing because it is not limited by challenges imposed by field conditions. Furthermore, automated tracking of scorpion movements allows for more efficient analysis and concurrent assessment of multiple subjects in similar environmental conditions. We plan to use this assay to systematically deduce the primary sensory cues used by animals as they return to their home shelters. Since scorpions more often returned to their shelters in lit conditions than in dark, it may be useful to displace scorpions from their shelters with the lights on, which would presumably prompt an immediate return path recordable by our software. An
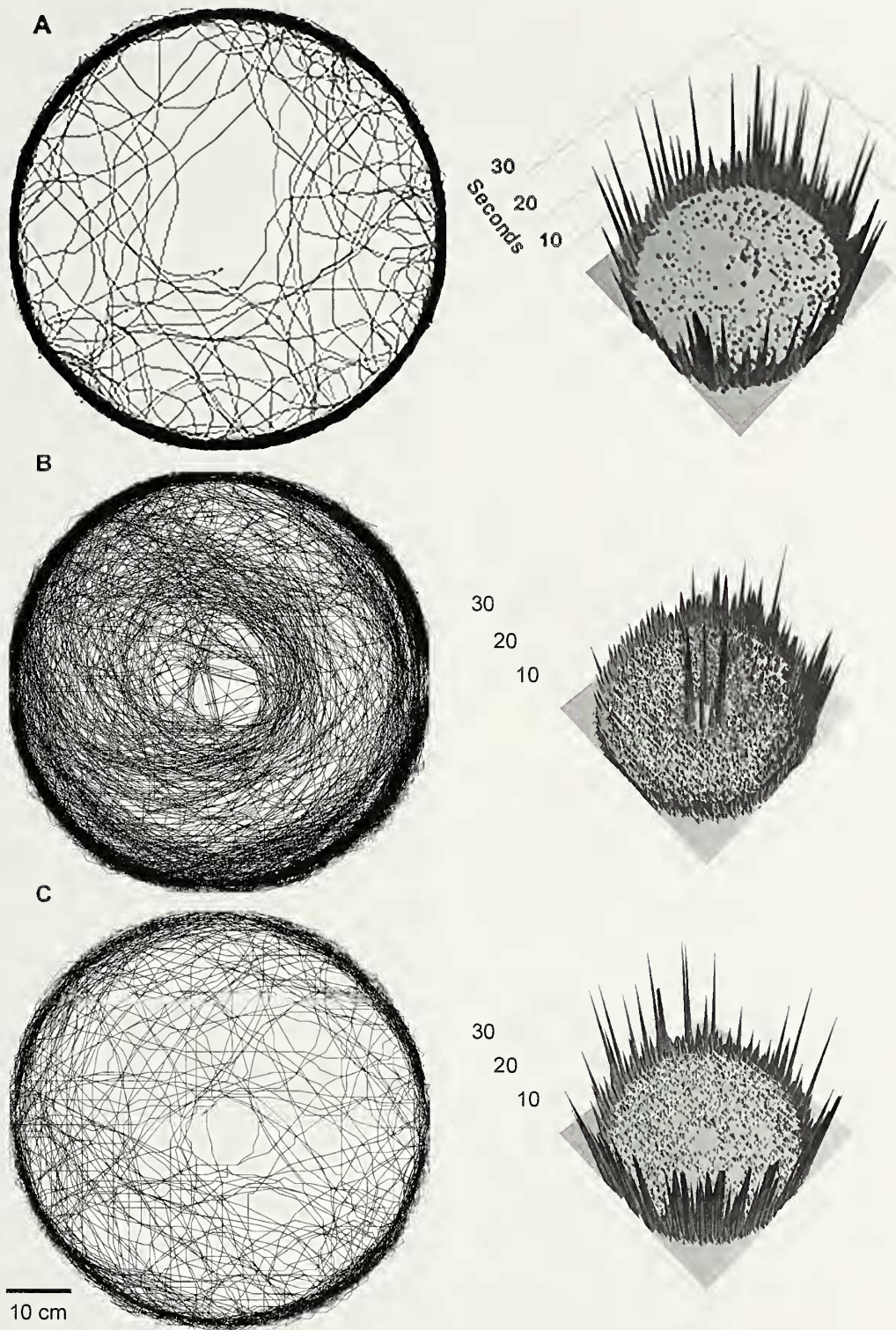
Figure 3.—Activity of three scorpions over a 7-hour dark period. A, B, and C represent individual scorpion data. Left panels: Path data collected from day 1 during the first 7 hours of darkness. The circle of relative absence in the center of each plot is the location of the shelter. In the second plot's center, the triangular plot pattern's vertices connect the three shelter entrances. Right panels: Z-axis values represent plot density measured in seconds of movement per measured unit area (0.648 cm^2).

alternative would be to change the on/off light cycles to last a much shorter period of time, which may induce homing behavior more often.

Scorpions might use several strategies to find their burrows. For example, because scorpion eyes are sensitive to very low light levels (Fleissner & Fleissner 2001), star patterns might be used as a homing reference. Even in our indoor arenas, the animals could have been using other sources of light within the room. However, the animals were filmed under IR light (which they cannot see; Camp & Gaffin 1999) and the cardboard

boxes blocked direct light from other sources (such as the LEDs on the video recorder).

Scorpions also have chemo- and mechanosensory sensilla on their tarsi and pectines that could be used to track self-made chemical deposits and/or footstep patterns back to their home burrows. Disrupting the sand around the artificial shelter while the animal is at the arena wall should help reveal the importance of these cues.

In previous studies, we have filmed animals under IR light in their native habitats as they made excursions from and to their home burrows (Gaffin 2011). Animals would often wait at their burrow threshold until induced to emerge by the vibrations caused by nearby moving arthropods. We also found we could coax animals to follow small wads of paper pulled past their burrows with thread. In another study, we coaxed an animal onto a rigid platform buried just beneath the sand surface. When we displaced the platform several centimeters, the scorpion's first movements were to a point where its burrow would have been had it not been displaced (Gaffin et al. 2012). This suggests that the animals may use a path integration mechanism for approximating the angle and distance to their burrow. Using such coaxing and displacement strategies in our in-lab assay should provide additional insights into the use of path integration. If true, this sets up the possibility that the animals might track familiar sensory cues experienced during the initial home foray established by path integration (Baddeley et al. 2012).

ACKNOWLEDGMENTS

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